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Antioxidants in Decelerating Diabetic Nephropathy

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1. Introduction

Diabetes mellitus (DM) is a group of metabolic diseases characterized with inappropriate hyperglycemia due to either a deficiency of insulin secretion or a combination of insulin resistance and inadequate insulin secretion (Masharani, 2008). Type 1 diabetes is caused by absolute deficiency of insulin secretion. Individuals at risk of developing this type of diabetes are found with serologic evidence of an autoimmune process occurring in the pancreatic islets and by genetic markers. In type 2 diabetes, it is a combination of resistance to insulin action and an inadequate compensatory insulin secretion response (American Diabetes Association, 2008). Diabetic nephropathy, one of the common complications of diabetes, has become the leading cause of end-stage renal failure in many countries (Chen et al., 2005). In general, about 1 out of 3 patients with type 1 or type 2 diabetes proceed to developing significant diabetic nephropathy (Zipp and Schelling, 2003). It is believed that the pathophysiologic mechanisms of renal disorder are similar in both types of diabetes (Kern et al., 1999). The pathogenesis and clinical course of diabetic nephropathy can be monitored by structural and hemodynamic changes. The earliest changes is an increase in glomerular filtration rate (GFR), also call "hyperfiltration" stage, which is followed by detectable glomerular lesions with normal albumin excretion rate. The next change is the development of microalbuminuria. Once microalbuminuria persist, both changes in glomerular structure, such as mesangial expansion and basement membrane thickening, and permeability happened, which is referred as "incipient nephropathy". Diabetic subjects with persistent microalbuminuria are at increased risk for "overt diabetic nephropathy". At this stage, prominent proteinuria, hypertension, and renal insufficiency progressed. The pathological findings in this stage are glomerular basement membrane (GBM) thickening, mesangial expansion and resulting in diffuse and/or nodular glomerulosclerosis, afferent and efferent arteriolar hyalinosis, and tubulointerstitial fibrosis (Cooper and Gilbert, 2003). After several years of persistent proteinuria, progression to end-stage renal disease will occur (Caramori and Mauer, 2001).

Advanced diabetic glomerulopathy is commonly characterized by diffuse glomerulosclerosis and may sometimes exhibit a distinctive morphological appearance, namely, the nodular form of glomerulosclerosis, as first described by Kimmelstiel and Wilson in 1936 (Kimmelstiel and Wilson, 1936; Kern et al., 1999). The stages of diabetic nephropathy are shown in Table 1 (Vora and Ibrahim, 2003).

Stage	Renal manifestation
1	Renal hyperfiltration (GFR↑) Renal hypertrophy
2	Silent stage Renal hyperfiltration (GFR*↑); Normal UAER*, blood pressure Early histologic changes: non-specific increase in basement membrane thickness, increase mesangial matrix
3	Microalbuminuria (UAER 30-300mg/24 h) or incipient nephropathy GFR may elevated or reduced into normal range. Histology: mesangial expansion, glomerular basement membrane thickening, arteriolar hyalinosis
4	Established or overt nephropathy (Proteinuria, nephrotic syndrome) GFR decline, Hypertension Histology: mesangial nodules (Kimmelstiel-Wilson lesions), tubulointerstitial fibrosis
5	ESRD***

* GFR, glomerular filtration rate
**UAER, urine albumin excretion rate
***ESRD, end stage renal disease

Table 1. Natural course of diabetic nephropathy in type 1 diabetes

The current strategies to treat diabetic nephropathy include intensive glycemic control, anti-hypertensive treatment with a particular focus on the interruption of renin-angiotensin-aldosterone system (RAS), restriction of dietary protein, and treatment of hyperlipidemia. There are several new approaches to the treatment of diabetic nephropathy based on an ever-growing mechanistic understanding of the causes of diabetic nephropathy by the specific pathogenic roles. These agents include pharmacologic inhibitors of advanced glycation end products (AGEs) formation, protein kinase C (PKC), oxidative stress, and transforming growth factor β (TGF- β) (Williams and Stanton, 2005).

2. Animal models of diabetes mellitus

Type 1 diabetes mellitus is typically an immune mediated destruction of the pancreatic

β cells. Type 2 diabetes mellitus is characterized by insulin resistance and insulin secretion impairment. Animal models have been used extensively in the field of diabetes study. The current available animal models of type 1 and type 2 diabetes are shown in Table 2 (Rees and Alcolado, 2005).

Type 1	BB (Bio breeding) rat Chinese hamster Celebes black ape Keeshond dog LETL (Long Evans Tokushima lean) rat New Zeland white rabbit NOD (non-obese diabetic) mouse Streptozotocin-induced rats
Type 2	CBA/Ca mouse db/db mouse Diabetic Torri rat GK (GotoKakizaki) rat Israeli sand rat KK mouse New Zeland obese mouse NSY (Nagoya-Shibata-Yasuda) mouse Ob/Ob mouse OLETF (Otsuka Long-Evans Tokushima fatty) rat Zucker rat

Table 2. Animal models of type 1 and 2 diabetes mellitus

3. The molecular mechanism of oxidative stress in diabetic nephropathy

There are four major biochemical pathways considered to lead to the development of diabetic complications associated with hyperglycemia, (1) the polyol pathway, glucose is converted to sorbitol and then metabolized to fructose. Advanced glycation end products (AGE) and reactive oxygen species (ROS) formation also occurs via this pathway, (2) the hexosamine pathway, fructose-6-phosphate is converted to glucosamine intermediates and the production of ROS is subsequently increased, (3) the protein kinase C (PKC) pathway, glucose is converted to glyceraldehyde-3-phosphate and leads to the formation of diacylglycerol (DAG). The elevation of intracellular DAG levels activate PKC, and then activate NADPH oxidase to induce ROS, (4) the formation of advanced glycation end products (AGEs), interaction of AGEs with the receptors of advanced glycation end-products (RAGE) results in ROS activation (Stirban et al., 2008; Shah et al., 2009; Forbes et al., 2008; Brownlee, 2005; Kanwar et al., 2008; Singh et al., 2011).

Increased oxidative stress has been a widely accepted participant in the development and progression of diabetes and its complications (Maritim et al., 2003). ROS are activated in glomerular mesangial and tubular epithelial cells by high glucose, AGE, and cytokines (Park et al., 1999). Hyperglycemia activates the glycolytic pathway and excess generation of mitochondrial ROS initiates a vicious circle by activating several signaling to increase protein kinase C (PKC), and stimulating NADPH oxidase to induce ROS generation (Johansen et al., 2005). Free radicals has been found to be formed disproportionately increase in diabetic subjects by glucose oxidation, nonenzymaticglycation of proteins, and then oxidative degradation of glycated proteins. Excessively amount of free radicals induce damage to cellular proteins, membrane lipids, nucleic acids, and then cell death (Maritim et al., 2003). Besides, increased ROS can cause vascular endothelium abnormalities, reacting directly with nitric oxide (NO) to produce cytotoxic peroxynitrite and increasing reactivity to vasoconstrictors and modification of extracellular matrix proteins (Schnackenberg, 2002). ROS can also damage endothelial cells indirectly by stimulating expression of various genes involved in inflammatory pathway (Baldwin, 1996). Previous study finds that high glucose induces ROS and then up-regulates TGF- β 1 and extracellular matrix (ECM) expression in the glomerular mesangial cell (Lee et al., 2003). There are also evidences that antioxidants can effectively inhibit high glucose induced TGF- β 1 and fibronectin up-regulation (Ha et al., 1997). Ha et al. (2002) reported that ROS mediate high glucose-induced activation of NF- κ B and NF- κ B dependent monocyte chemoattractant protein (MCP)-1 expression. NF- κ B, a nuclear transcription factor, can initiate the transcription of genes associated with inflammatory response. It is induced by various cell stress-associated stimuli including growth factors, vasoactive agents, cytokines, and oxidative stress (Kuhad and Chopra, 2009). Advanced glycation end products induced by hyperglycemia stimulate NF- κ B activation, which sustains the activation of NF- κ B in diabetes (Gao et al., 2006). Increased steady-state mRNA levels of inflammatory genes have been shown to associate with interstitial fibrosis and progressive human diabetic nephropathy (Kuhad and Chopra, 2009).

TGF- β plays an important role in the development of renal hypertrophy and accumulation of extracellular matrix (ECM) components in diabetes mellitus (Wolf and Ziyadeh, 1999). The expression of TGF- β was found increased in diabetic nephropathy of experimental animals and in humans (Park et al., 1997; Yamamoto et al., 1993; Sharma et al., 1997; Shankland et al., 1994). Treatment with anti-TGF- β antibody has been documented that it attenuated the effect of high glucose induced cellular hypertrophy *in vitro* and in streptozotocin-induced diabetic mice (Wolf et al., 1992; Ziyadeh et al., 1994; Sharma et al., 1996). TGF- β is also the key regulator of ECM remodeling in mesangium causing mesangial expansion and inducing the process of epithelial-mesenchymal transition (EMT) causing tubulointerstitial fibrosis (Ziyadeh et al., 2000; Oldfield et al., 2001). As the accumulation of ECM and persistence of tubulointerstitial fibrosis, the renal function progress to end-stage renal disease (ESRD). The relation between oxidative stress and diabetic nephropathy are shown in Figure 1 (Shah et al, 2007).

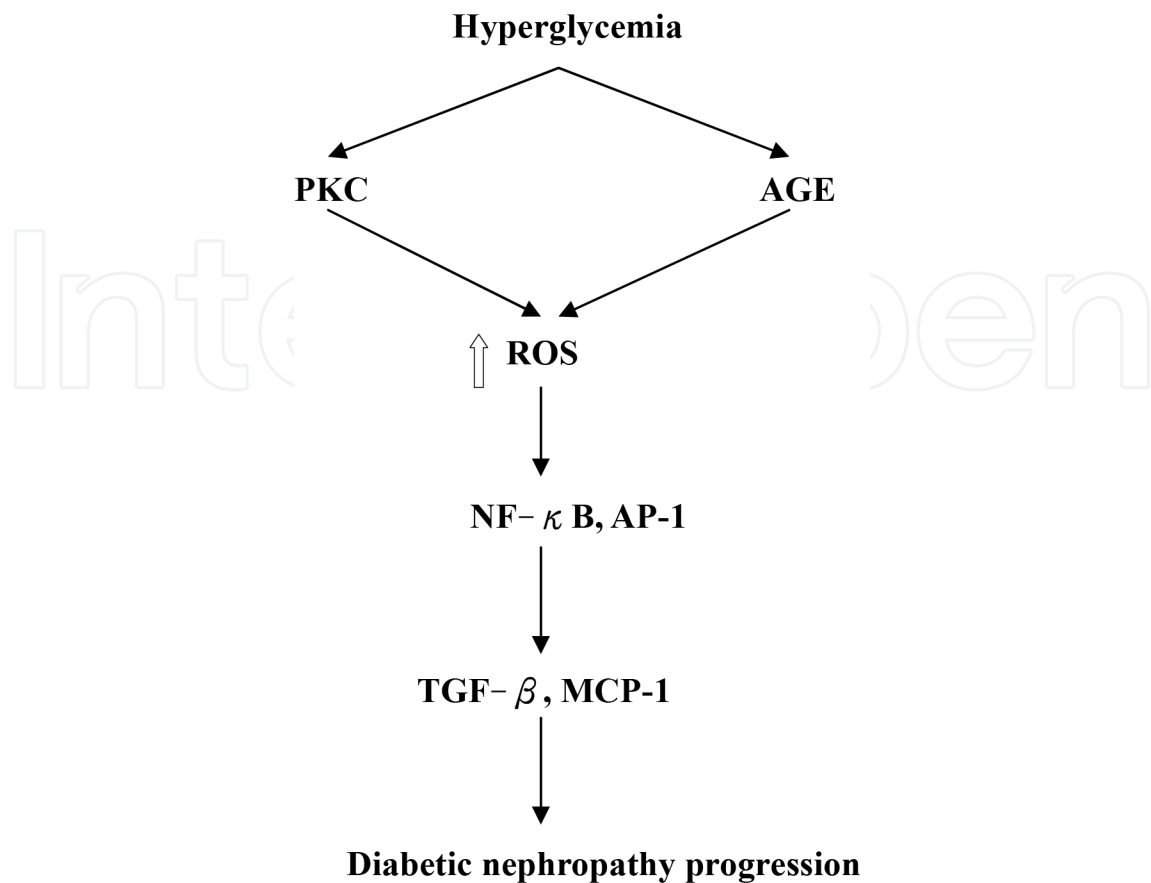


Figure 1. The relation between oxidative stress and diabetic nephropathy* *PKC, protein kinase C; AGE, advanced glycation end products; ROS, reactive oxygen species; NF-κB, nuclear factor-kappa B; AP-1, activator protein-1; TGF-β, transforming growth factor-beta; MCP-1, monocyte chemotactic protein-1

4. Improvement of antioxidative status in diabetic nephropathy

There are many evidences suggest that ROS play an important role in the pathogenesis of diabetic nephropathy (Rosen et al., 2001). To prevent the development and progression of diabetic nephropathy, it would be effective in combing the strategies to prevent overproduction of ROS and to increase the removal of preformed ROS. (Ha et al., 2008). Some natural products were proved to possess the ability to decelerate diabetic nephropathy via reducing oxidative status. The flower of *Hibiscus sabdariffa* Linnaeus calyx (family Malvaceae, local name Karkaday) is commonly used in cold and hot beverages and as a supplement due to its perceived potential of health benefits. The flower extract has been reported to decrease blood pressure, and have antitumor characteristics as well as immune-modulating and anti-leukemic effects (Haji Faraji and Haji Tarkhani, 1999; Tseng et al., 2000). *Hibiscus sabdariffa* L. extract contains polyphenolic acids, flavonoids, protocatechuic acid (PCA) and anthocyanins. *Hibiscus sabdariffa* L. extract has been found to contain various polyphenols and was shown to have antioxidative potential to inhibit the development of atherosclerosis in cho-

lesterol-fed rabbits, LDL oxidation and ox-LDL-mediated macrophage apoptosis (Chen et al., 2003; Chang et al., 2006). Wang et al. (2009) demonstrated that aqueous extract of *Hibiscus sabdariffa* L. (HSE) is capable of increasing catalase and glutathione activities significantly in diabetic kidney. In histological examination, HSE improves hydropic change of renal proximal convoluted tubules in diabetic rats. HSE was also revealed to up-regulate Akt/Bad/14-3-3^σ and NF-κB-mediated transcription in diabetic nephropathy. Luteolin is a plant-derived flavonoid, it has various biological activities including anti-inflammatory (Jang et al., 2008), antimutagenic, and antitumorigenic properties (Ross and Kasum, 2002). It also possesses direct antioxidant activity (L'opez-L'azaro, 2009), and may be useful in treatment of many chronic disease associated with oxidative stress, such as cardiovascular diseases (McCord, 1985; Jeroudi et al., 1994), liver diseases (Comporti, 1985; Poli et al., 1987), diabetes (Oberley, 1988), and aging (Harman, 1981). Wang et al. (2011) demonstrated that luteolin has protecting effect against development of diabetic nephropathy by changing the superoxide dismutase (SOD) activity, the malondialdehyde (MDA) content, and expression of Heme Oxygenase-1 (HO-1) protein.

On the other hand, some evidences show the exogenous or endogenous antioxidants also can reduce diabetic nephropathy. Oxidative stress via nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and vascular endothelial growth factor (VEGF) pathway are documented to play important roles in the development of diabetic nephropathy. Nam et al. (2009) showed the effects of apocynin, a NADPH oxidase inhibitor, on diabetic nephropathy. They found that apocynin can not significantly decrease serum glucose levels but reduce urinary protein and albumin excretions. It is improved in glomerular and mesangial expansion as the apocynin treatment. Apocynin also decreased glomerular VEGF expression and reduced the concentration of 24 h urinary 8-OHdG and MDA. Additionally, Lee et al. (2005) demonstrated that antioxidant taurine prevented glomerular hypertrophy, mesangial expansion, and proteinuria in diabetic rats. Overexpression of catalytic antioxidants was also shown to protect against diabetic injury in several transgenic animals. Craven et al. (2001) showed that diabetic mice transgenic for Cu/Zn SOD had significantly lower urinary albumin excretion, glomerular hypertrophy, and glomerular expression of TGF-β1 and collagen IV protein compared to non-transgenic mice. Hamada et al. (2007) demonstrated that overexpression a small antioxidant, thioredoxin 1, effectively inhibited 8-OHdG in the kidney, albuminuria, mesangial expansion, and tubular injury in diabetic mice. Du et al. (2003) found that overexpression of MnSOD in bovine aortic endothelial cells prevented high glucose-induced activation of PKC, NK-κB, hexosamine, and advanced glycation end product (AGE) pathways. Brezniceanu et al. (2007) demonstrated that renal catalase overexpression in db/db mice attenuated ROS generation, angiotensinogen, proapoptotic gene expression and apoptosis in the kidneys of diabetic mice *in vivo*.

Although strict glycemic control is very important in DM patients, many of the current standard therapeutic approaches may also ameliorate oxidative stress as pleiotropic effects (Singh et al., 2011), such as angiotensin-2 converting enzyme (ACE) inhibitors (Kobayashi et al., 2006), angiotensin-2 receptor blockers (ARB) (Ogawa et al., 2006) and aldosterone blockers (spironolactone) (Takebayashi et al., 2006). They activate eNOS to increase bioavailability

of nitric oxide, inhibit synthesis of angiotensin 2 and TGF- β and to decelerate or prevent tubulointerstitial fibrosis in diabetic nephropathy, accompanied with control of systemic and intrarenal blood pressure. Cilostazol is a specific inhibitor of phosphodiesterase 3 (PDE 3). Its major effects are prevention of platelet aggregation and dilation of blood vessels via an increase in tissue cAMP levels (Matsumoto et al., 2005). Cilostazol was shown to inhibit vascular smooth muscle cell proliferation *in vitro* as well as suppress neointimal formation in balloon-injured rat carotid arteries due to its antiplatelet and vasodilator properties (Takahashi et al., 1992; Ishizaka et al., 1999). Our previous study showed that cilostazol decreases reactive oxygen species activity significantly in the kidneys of diabetic rats and improves urine albumin/creatinine ratio. Cilostazol also can improve the diabetes-caused increasing glomerular size, TGF- β , and NF- κ B in early diabetic nephropathy (Lee et al., 2010). The lipid-lowering agents such as statins, which can inhibit HMG-CoA reductase to be demonstrated to activate eNOS, maintain glomerular filtration rate and renal cortical blood flow, and further to ameliorate glomerular lesions (Usui et al., 2003; Endres and Laufs, 2004). Benfotiamine was used in the treatment of diabetic nephropathy, it was also demonstrated to reduce ROS formation and may decrease hyperfiltration and proteinuria in patients with diabetic nephropathy (Babaei-Jadidi et al., 2003). Potential therapies in these ideal antioxidants would influence the pathways of ROS generation to decelerate diabetic nephropathy.

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